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Environmental monitoring has evolved over the last ten years toward providing data more useful for exposure and risk assessment. The objective of many monitoring studies in the 1960's and 1970's was to monitor concentrations of pollutants including environmental mutagens at ambient locations, such as roof tops and in large bodies of water, where the pollutants would be well mixed and represent a homogeneous sample. In the 1980's, a number of studies focused on assessing the emission of mutagens from various sources. Now the emphasis has shifted to monitoring human exposure to environmental mutagens and to understanding which sources and factors lead to increased exposure and potential cancer risk. This chapter will briefly review advances in genetic bioassay methods for environmental monitoring and will focus on approaches to integrating genetic bioassay methods with environmental monitoring studies.

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Chapter 13

Environmental Monitoring using Genetic Bioassays

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INTRODUCTION

Monitoring, particularly environmental monitoring, refers to measuring a parameter in the environment over time and/or across various locations in order to keep track of, regulate, or control that parameter. The development of short-term genetic bioassays in the mid-1970's led rapidly to the use of these assays in environmental monitoring. The essential elements of these bioassays that facilitated their application to environmental monitoring was several-fold, including their relative simplicity, rapidity, low cost, and the small sample size requirements. The genetic bioassay used most widely has been the Salmonella typhimurium plate-incorporation assay that Bruce Ames described in 1975 and validated as a bioassay to screen for carcinogens (see Chapter 9 and 12). This bioassay was adopted rapidly across the world and applied to environmental studies of complex mixtures (see Chapter 9). Within two years after Ames's description of this bacterial mutagenesis bioassay, scientists in the U.S., Japan, and Germany reported conducting air pollution monitoring studies in which this bioassay was used to detect and quantitate the mutagenic activity associated with air particulate matter. In 1978, a Symposium on the Application of Short-Term Bioassays to the Analysis of Complex Environmental Mixtures reported on the use of short-term genetic bioassays in monitoring air, water, industrial effluents, and commercial products (e.g. synthetic petroleum products, cigarette smoke, and food) (Waters et al. 1979).

Environmental monitoring using genetic bioassay methods is accomplished through either collecting environmental samples in the field, which are analyzed later in the laboratory, or by taking bioassays to the field for "in situ" monitoring where the exposure occurs directly in the environment. Genetic bioassays that have been applied to "in situ" monitoring include plant, animal, and aquatic organisms using either indigenous or laboratory organisms. The genetic knowledge of plant systems and the ease with which they can be adapted to field studies led to the use of plants, especially Tradescantia, for air pollution studies conducted in the 1970's (Waters et al. 1979). Recent environmental concern surrounding hazardous waste sites has led to the use of a wider range of organisms, including indigenous animals, in field monitoring studies. A wider range of "in situ" monitoring methods using genetic bioassays are now being developed. The first approach of collecting environmental samples in the field for laboratory bioassays has been applied more extensively to environmental monitoring studies and will be used for most of the examples cited in this chapter.

Environmental monitoring has evolved over the last ten years toward providing data more useful for exposure and risk assessment. The objective of many monitoring studies in the 1960's and 1970's was to monitor concentrations of pollutants including environmental mutagens at ambient locations, such as roof tops and in large bodies of water, where the pollutants would be well mixed and represent a homogeneous sample. In the 1980's, a number of studies focused on assessing the emission of mutagens from various sources. Now the emphasis has shifted to

monitoring human exposure to environmental mutagens and to understanding which sources and factors lead to increased exposure and potential cancer risk. This chapter will briefly review advances in genetic bioassay methods for environmental monitoring and will focus on approaches to integrating genetic bioassay methods with environmental monitoring studies.

ADVANCES IN GENETIC BIOASSAY METHODS FOR ENVIRONMENTAL MONITORING

Assay Systems

Although many of the genetic assay systems described in Chapter 12 could be applied to environmental monitoring, in practice a limited number of these systems are used. Environmental monitoring studies require that a relatively large number of measurements be made over time, across locations, or under different conditions. This data requirement imposes the use of the most sensitive, quantitative, rapid, and inexpensive of the genetic bioassays. Of the mutation assays available, the Ames plate-incorporation assay and other Salmonella typhimurium assays (e.g. preincubation, forward mutations, etc.) have been most useful for monitoring air, water, waste, or other environmental samples. Of the cytogenetic assays, recent studies have selected the micronucleus test as a simple and rapid cytogenetic assay that is often applied to "in situ" monitoring studies. The sister chromatid exchange assay was initially the most commonly used cytogenetic assay used in monitoring studies due to its sensitivity when

compared to chromosomal aberration assays. Very few of the assays detecting DNA effects have met the requirements imposed by environmental monitoring assays; however, an assay that measures induction of λ prophage in E. coli WP2 (T. Rossman et al. in Waters et al. 1985) and the umu-test for induction of SOS response (T. M. Ong et al. in [illegible] et al. 1987) have been applied to environmental monitoring st

Microassay Methods

The relatively small sample mass requirements (milligram quantities) of short-term genetic bioassays was one of the important attributes that facilitated their application to environmental monitoring. Recent advances in exposure monitoring have resulted in the need for assay methods that could be applied to even smaller, microgram quantities of environmental samples. Microsuspension bacterial mutation assays have been developed to meet these needs through simple modifications of the Salmonella typhimurium preincubation assay in which the assay is conducted in a 10-100 fold smaller volume (Kado et al. 1986). Microsuspension assays have been applied to indoor air pollution monitoring, human exposure monitoring, and in monitoring trends in air pollution over relatively short time periods.

MONITORING EMISSIONS AND EFFLUENTS FROM SOURCES

The application of bioassay methods to monitoring the mutagenicity of industrial and other source emissions and effluents was one of the

earliest applications of genetic bioassays (Waters et al. 1979). Short-term bioassays have proven useful in evaluating the effects of various engines, fuels, and control technologies on the mutagenicity of emissions from combustion sources (Lewtas, 1982). Emissions from combustion sources were collected, extracted, and tested in the Ames assay in order to quantify and compare the magnitude of mutagenic emissions. Aqueous effluents from various industrial sources have been collected from the plant outlet pipes in order to monitor the emissions of mutagens from those industries. These earliest studies showed that bacterial mutagenicity assays could readily detect the emission of mutagens from many of these sources.

Mutagenic emission factors are used to compare the mutagenicity of emissions from different sources as shown in Figure 1.
To compare the mutagenicity of emissions from various sources, it is critical to compare mutagenic emission factors on the same basis (e.g. fuel consumption, distance driven, energy consumption, or yield). Mutagenic emission factors of mobile sources are expressed as mutagenic activity per kilometer. The mutagenic emission factors from stationary sources are expressed as the basis of fuel or energy consumption (revertants/kg fuel or per joule heat). This direct comparative analysis of mutagenic emission factors provides a simple means of evaluating various sources. Typical mutagenic emission factors for stationary and mobile sources are shown in Figure 1.

The organic emission factor may vary more than the mutagenicity or carcinogenicity of the organics associated with emissions; and, therefore, in the final analysis has a greater impact on the emission factors and relative potential carcinogenic risk from those sources. Combustion emissions are a good example of this. The Ames Salmonella typhimurium mutagenesis assay showed approximately two orders of magnitude difference in mutagenic activity (rev/μg) from the weakest to the most potent combustion emission sample. The emission rates (rev/km or rev/J) for the particle-bound organics, however, differed in some cases by as much as five orders of magnitude (10^5) (Lewtas, 1985).

The Ames Salmonella/microsomal assay has found extensive application in evaluations of the mutagenic activity of aqueous effluents from municipal and industrial wastewaters. Industrial effluent studies have concentrated on either specific manufacturing process types, such as coal gasification processes and textile mill effluents, or surveyed the mutagenicity of effluents discharged from a number of industrial process types. Generally, aqueous effluents must be concentrated prior to mutagenicity testing. Therefore, to compare the mutagenic emission rate from effluents, the mutagenic activity of the concentrated organics (e.g. in revertants per milligram organics) is multiplied by the micrograms of organics per volume of effluent (organic discharge rate) to determine the emission rate per volume of effluent (revertants per liter). The range of mutagenic emissions from various industrial effluents has ranged from less than 100 rev/liter to over 100,000 rev/liter as shown in Fig. 1.

MONITORING THE AMBIENT ENVIRONMENT

Ambient air particles contain extractable organic matter that, over 45 years ago, was first shown to be carcinogenic in rodents and later shown to induce oncogenic transformations in mammalian cells in culture. These bioassays require relatively large quantities of airborne particles and take many months to yield data that are often difficult to quantitate. The development of the Ames mutagenesis bioassay provided a bioassay for potential carcinogenic activity that was simpler, more sensitive, faster than rodent carcinogenicity assays, and that could be applied to air samples collected by conventional air monitoring methods. Studies in several countries showed that the extractable organics from air particles were mutagenic in the Ames bioassay. These studies showed that mutagenic activity was concentrated in the smaller particles (Figure 2) and was more concentrated at industrial and urbanized sites when compared to rural sites. Monitoring studies of trends in the concentration of mutagenic activity over time demonstrated higher concentrations of mutagens in the winter months and during certain times of the day related to traffic (Figure 3). Evidence that the airborne mutagenic activity was emitted from specific combustion emission sources was based on studies that showed the concentration of mutagenic activity was highest downwind from combustion sources as shown in Figure 4. Bioassay studies of the organics emitted from a variety of combustion sources (e.g. automobile and residential oil emissions) confirmed that mutagens are emitted from these sources.

Drinking water is the other major ambient environment through which humans are exposed to mutagens and carcinogens. Chemical analysis has shown that drinking water contains from hundreds to thousands of chemicals in low concentrations. Many of these chemicals result from disinfection of drinking water with chlorine. The first such chemicals identified were the trihalomethanes. The significance of these observations was increased by the finding that chloroform is carcinogenic in rats and mice. Results from animal studies were subsequently supported by a number of studies indicating a correlation between drinking water chlorination and gastrointestinal- and urinary-tract cancer mortality. In addition, drinking water concentrates and chemicals found in drinking water have been identified as mutagens in the Ames test. It is now thought that the trihalomethanes represent only a fraction of the products of chlorination. Many substances produced by chlorination of humic and fulvic acids and isolated from water remain to be identified.

EXPOSURE ASSESSMENT

Total human exposure assessment methods, developed to measure exposure to pollutants by all routes of exposure, show that for many pollutants, exposure via the air pathway is the most important. Sexton and Ryan (1988) in a recent review of human exposure to air pollution, emphasize that indoor exposures make a major contribution to the total human exposure to airborne particulate matter. In order to assess human exposure, the exposure in specific microenvironments must be

characterized as well as the fraction of time spent in each microenvironment. Two other approaches to assessing total exposure have been used: personal exposure monitoring and biological monitoring using body fluids, cells, or tissues (discussed in Chapter 14).

Monitoring Microenvironments

Monitoring exposure in various microenvironments (i.e., automobiles, homes, and work places) and determining the time spent in each has been used to determine total human exposure to environmental pollutants. The concentration of pollutants in indoor microenvironments is often higher than outdoors due to numerous indoor sources and the small mixing volume of air. Because homes have small air volume and low air exchange rates, indoor air samplers are limited to sampling less than 10% of the total volume per hour to prevent disturbing the normal air flow and concentrations. The microgram quantities of mass collected in indoor monitoring studies are inadequate for most bioassays. Microsuspension mutagenicity bioassay methods make it possible to detect and quantitate genotoxic activity with these sampling conditions.

In environmental monitoring studies, combustion sources have been found to make a major contribution to indoor mutagenicity if those sources were unvented. The highest concentration of mutagenic activity observed indoors or outdoors has been in indoor environments where tobacco smokers are present. Unvented kerosene heaters also resulted in elevated levels of mutagenic activity. Environmental monitoring studies

using genetic bioassay analysis of indoor air particulate matter have identified environmental tobacco smoke, emitted as sidestream smoke, to be the major source of mutagens associated with airborne particles indoors as shown in Figure 5.

Personal Exposure Monitoring

Until very recently, it has not been possible to couple genetic bioassays to personal exposure monitoring. The development of sampling and bioassay methods for application to personal monitoring is currently in progress. The development of such methods will result in a better understanding of the external exposure to genotoxic agents. Exposure monitoring can also be approached from the biomonitoring of human populations as discussed in Chapter 14. The combination of environmental and personal exposure monitoring with human population monitoring yields the optimal information for understanding the relationship between exposure, dose, and effects.

SOURCE APPORTIONMENT

Source apportionment refers to the determination of the contribution of specific sources to the pollutants found in an ambient mixture. These methods have been used widely in the air pollution studies that are described here, but similar methods could be applied to other environmental media.

The relative contribution of various sources to the concentration of airborne particles, organics, or mutagenic activity has been estimated using either dispersion modeling, receptor modeling, or some combination of these methods. Sufficient data are now available to apply modeling methods to estimate the contribution of various combustion and industrial sources to the mutagenic activity associated with the organics from airborne particles. A recent review summarizes the available mutagenicity and mutagenic emission factors from many sources and applies several different modeling methods to the data to estimate the contribution of these sources to the mutagenicity in ambient air (Lewtas et al. in press).

Dispersion Modeling

Dispersion modeling of mutagens requires either 1) a data base of mutagenic emission factors for each source that are input directly into air dispersion models or 2) mutagenicity data for each source that are applied to a parameter such as air particle concentrations determined through dispersion modeling. These two approaches have been used to estimate the concentration of mutagenic activity in urban air and the relative contribution from various sources (Lewtas et al. in press). Environmental monitoring using the Ames bioassay has been used to determine the emission factors as described above (see monitoring source emissions) and to monitor the concentration of mutagens observed in the ambient air. Dispersion modeling of urban air pollution sources showed that residential area sources, such as woodstoves, and mobile sources,

such as automobiles, make the largest contribution to the mutagens associated with air particulate matter as shown in Figure 6.

Receptor Modeling

Receptor modeling requires ambient monitoring of mutagenicity and tracer elements or compounds that are markers for the suspected sources of mutagens (Gordon, 1988). A multiple linear regression form of receptor modeling has been used to determine the sources of the mutagenicity of fine-particle ambient aerosol samples collected during the winter in several residential and urban area. In these studies, virtually all the mutagenicity could be accounted for by woodsmoke and motor vehicle emissions. Woodsmoke was found to be the greater contributor to the average ambient concentrations of both extractable organics and mutagenicity. The mutagenic potency (revertants per microgram) of extractable organics traced to motor vehicles, however, was three times greater than that with a woodsmoke origin as shown in Figure 7.

THE FUTURE OF GENETIC BIOASSAYS IN INTEGRATED ENVIRONMENTAL RISK ASSESSMENT STUDIES

Short-term genetic bioassays are being considered an integral part of increasingly more environmental assessment studies aimed at cancer or genetic risk assessment. No reasonable set of chemical analyses can fully characterize or monitor all of the potential genotoxic agents in

very complex mixtures (see Chapter 9). The use of short-term genetic bioassays complements chemical analysis to directly provide data on the genotoxic activity of complex mixtures. A series of case studies describing integrated assessments is found in Waters et al., 1982, 1985. These studies include studies of indoor and outdoor air pollution, industrial effluents, and occupational environments.

An important aspect of future environmental assessment studies using genetic bioassays should be the focus on total human exposure assessments where human monitoring methods discussed in Chapter 14 are combined with the environmental and personal exposure monitoring methods described in this chapter. Integrated multidisciplinary studies of this scope will make possible direct measurement of exposure, dosimetry, and genetic effects. An example of the use of biomonitoring in both environmental and human exposure is a study being conducted to assess exposure and lung cancer risk in a population in Xuan Wei Provence in China (Mumford et al., in press). This study involves three different cohorts exposed to combustion emissions in their home from either smoky coal, smokeless coal or wood. Biomonitoring of the air was conducted using bacterial genetic bioassays as described in this chapter. Mutagenesis studies in mammalian cells and animal tumor studies are being used to assess the comparative cancer potency of these three emissions. Human biomonitoring of DNA adducts in tissues from exposed individuals are being assessed as well as the cancer mortality in the populations. The combined use of genetic toxicology methods to assess environmental and human exposure, dosimetry and genotoxic effects

provides both powerful and practical methods for assessment of human risk from exposure to genotoxic agents.

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FIGURE LEGEND

Figure 1. Typical mutagenic emission factors for stationary sources (revertants/mega joule), mobile sources (revertants/kilometer), and industrial effluents (revertants/liter) (Lewtas, 1985; Holmberg and Ahlborg, 1983; Lewtas, 1988; L. McGeorge in Waters et al., 1985).

Figure 2. The effect of size on the mutagenic activity of airborne particles (Lewtas-Huisingh in Waters et al, 1981).

Figure 3. Monitoring the concentration of mutagens over time of year (Alfheim in Holmberg and Ahlborg, 1983) and time of day (Kado et al., 1986).

Figure 4. Relationship between wind direction and mutagenic activity (Lewtas-Huisingh in Waters et al., 1981; B. Commoner in Waters et al., 1979).

Figure 5. Monitoring the concentration of mutagenic activity indoors in relation to the presence of specific sources (Lewtas, 1987).

Figure 6. Contribution of various sources to airborne mutagenicity as determined from dispersion modeling (Lewtas et al., in press).

Figure 7. The relationship between mutagenic potency and contribution of woodsmoke and mobile sources to airborne mutagenicity as determined from receptor modeling (Lewis et al., 1988).

Typical Mutagenic Emission Factors

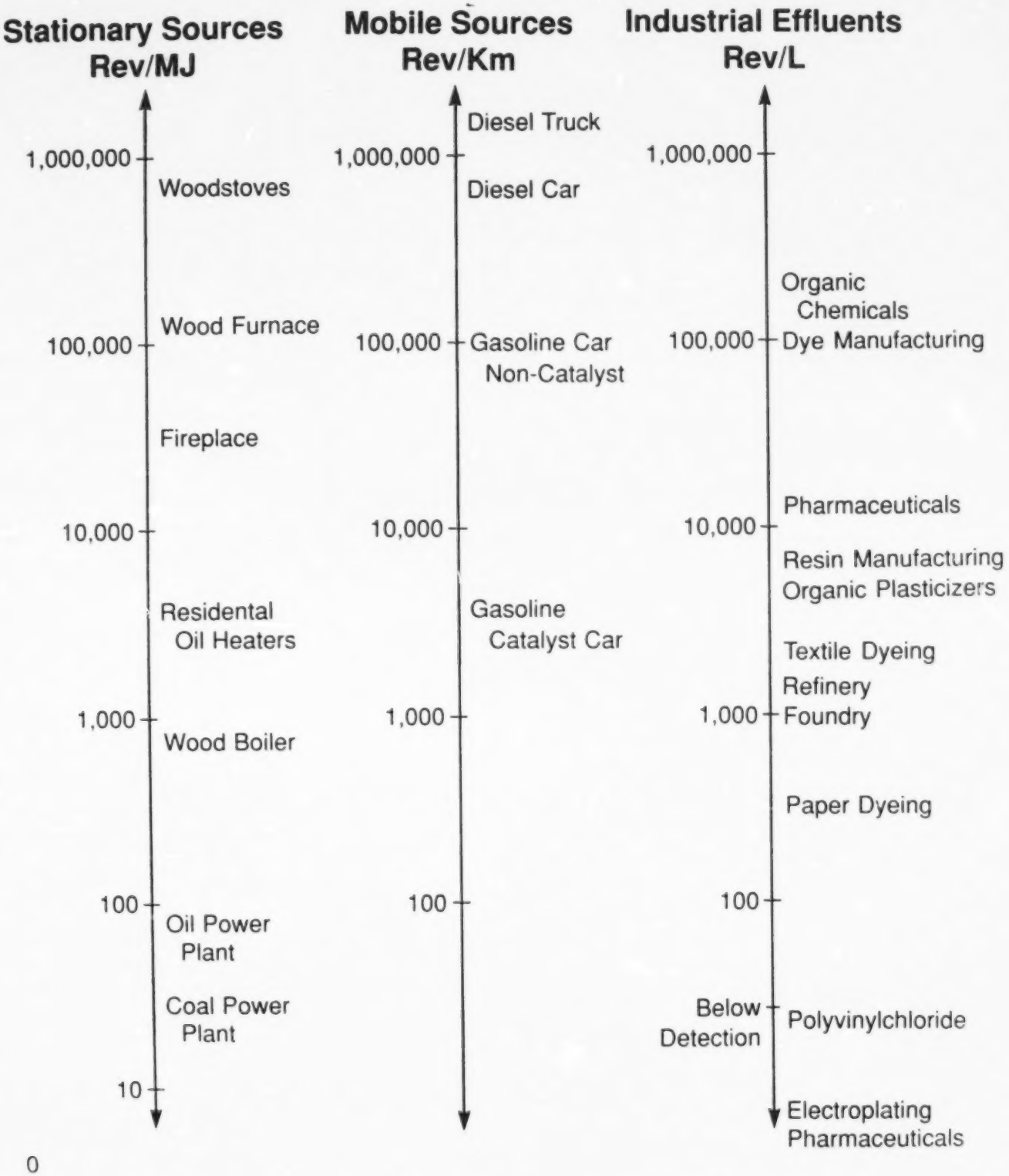


Fig. 1

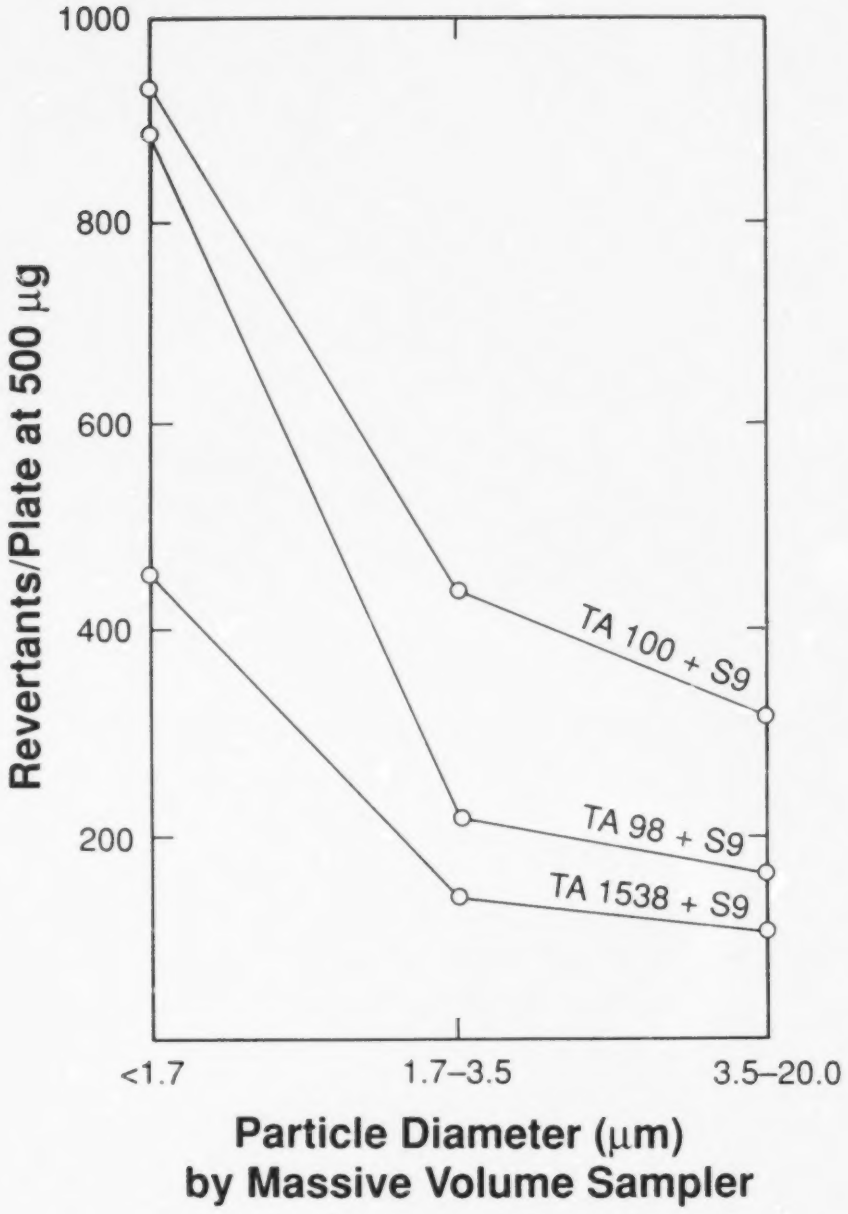
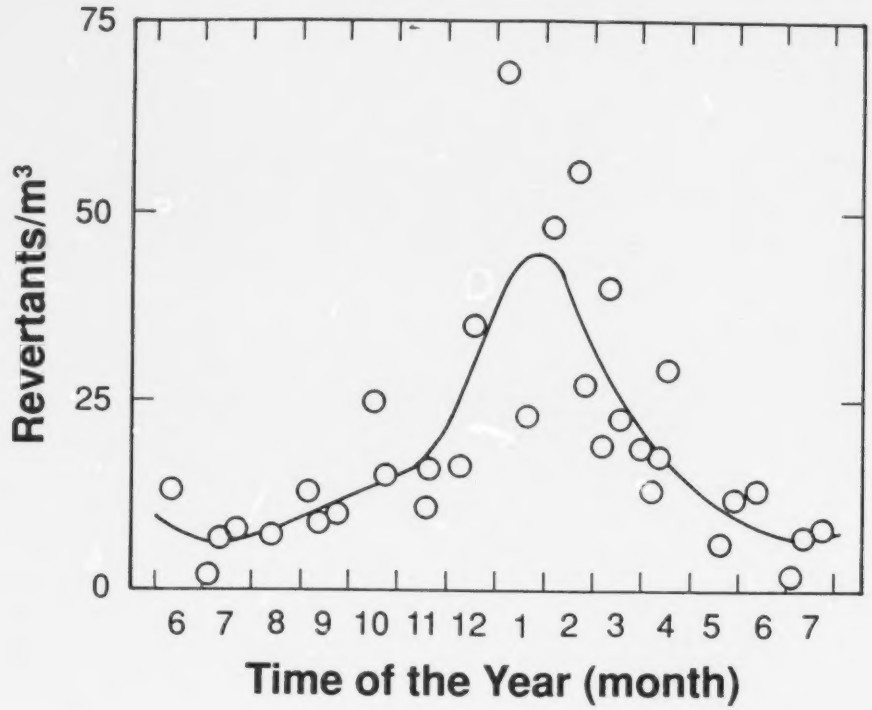

1000
800
600
400
200
Revertants/Plate at 500 µg
TA 100 + S9
TA 98 + S9
TA 1538 + S9
<1.7
1.7–3.5
3.5–20.0
Particle Diameter (µm)
by Massive Volume Sampler

Fig. 2


75
50
25
0
Revertants/m³
6 7 8 9 10 11 12 1 2 3 4 5 6 7
Time of the Year (month)

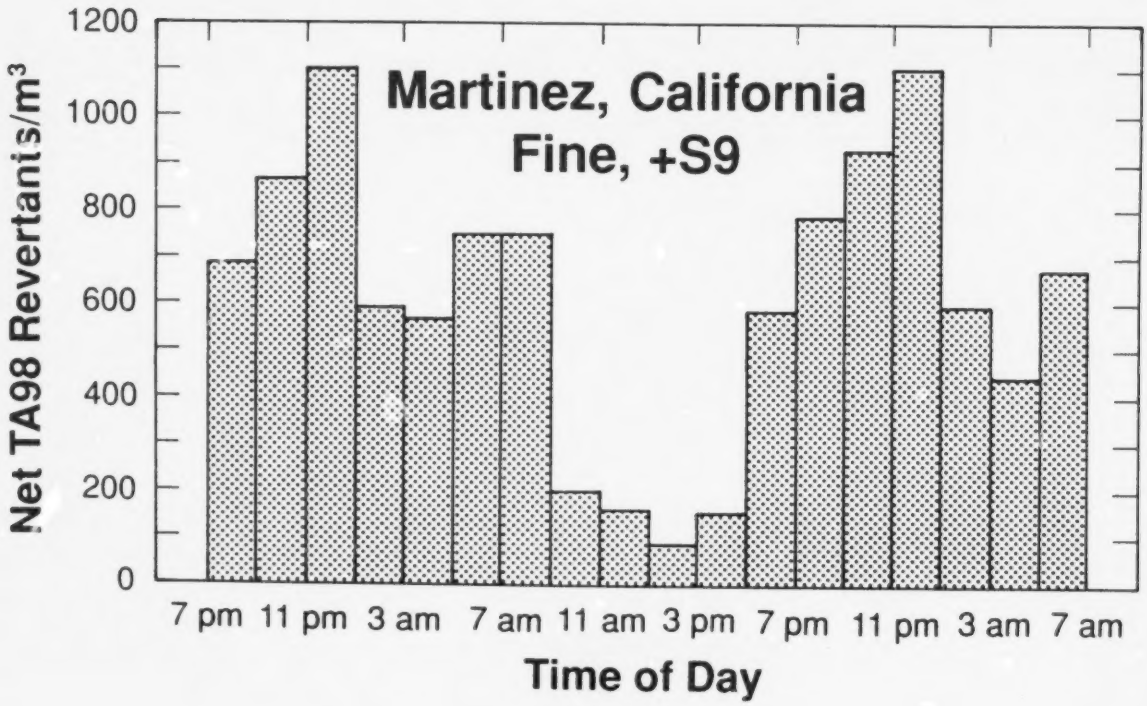
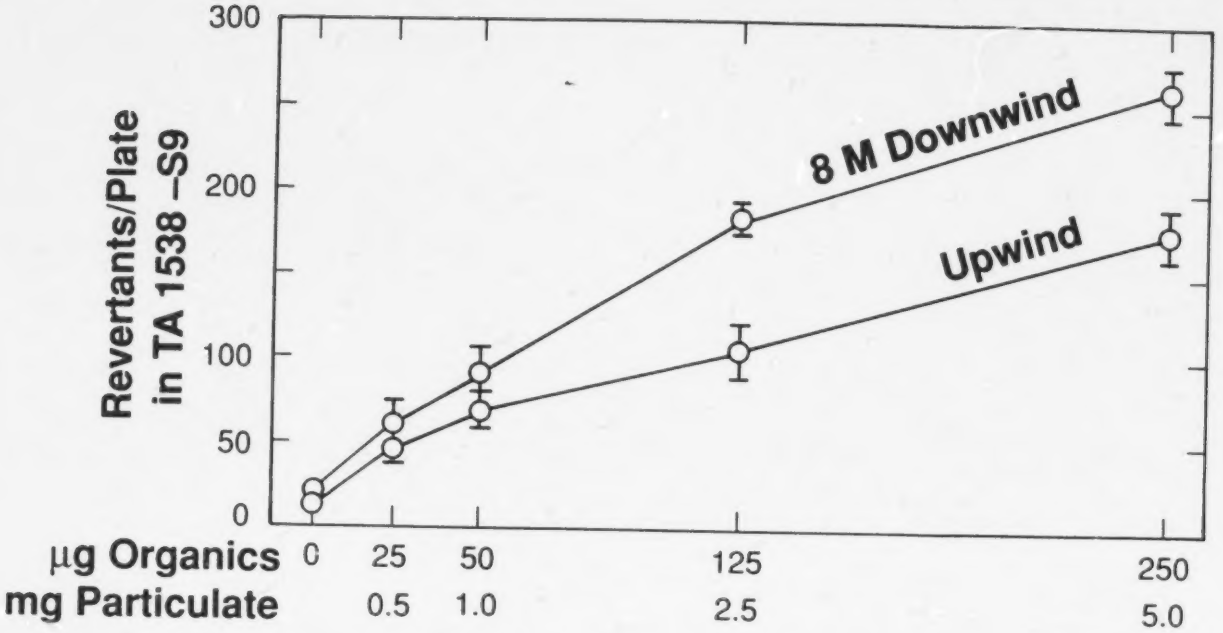

Martinez, California
Fine, +S9
1200
1000
800
600
400
200
0
Net TA98 Revertants/m³
7 pm 11 pm 3 am 7 am 11 am 3 pm 7 pm 11 pm 3 am 7 am
Time of Day

Fig. 3


300
200
100
50
0
Revertants/Plate
in TA 1538 –S9
8 M Downwind
Upwind
µg Organics 0 25 50 125 250
mg Particulate 0.5 1.0 2.5 5.0

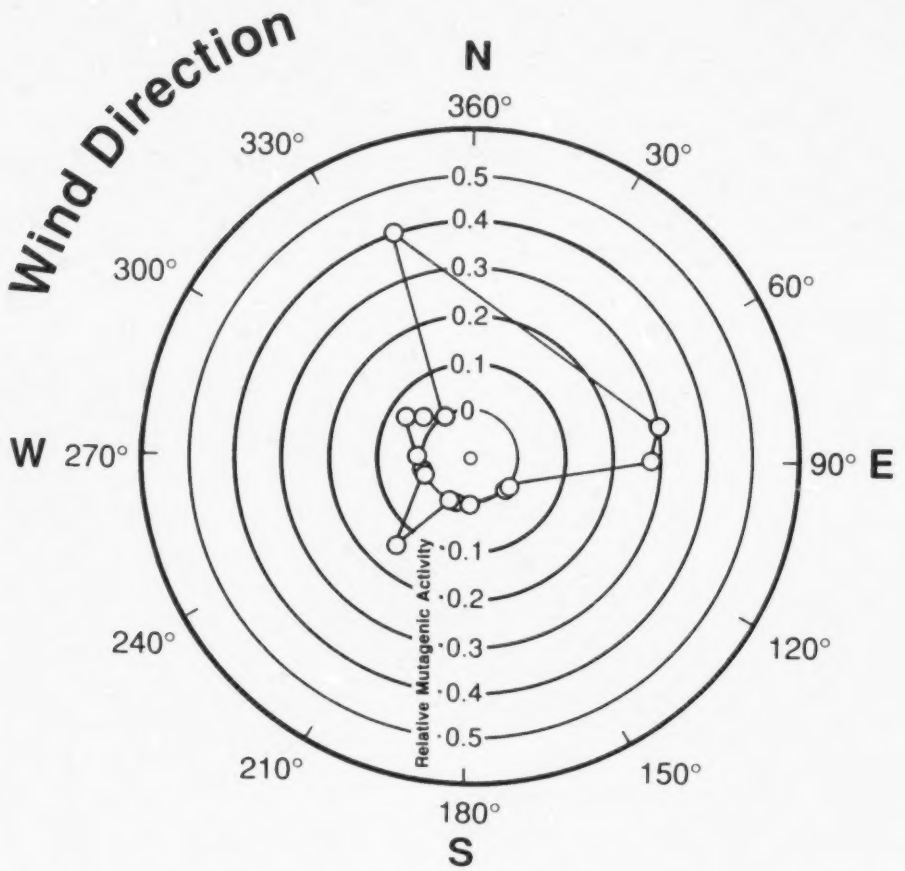

Wind Direction
N
360°
30°
60°
90° E
120°
150°
180°
S
210°
240°
W 270°
300°
330°
0.5
0.4
0.3
0.2
0.1
0
0.1
0.2
0.3
0.4
0.5
Relative Mutagenic Activity

Fig. 4

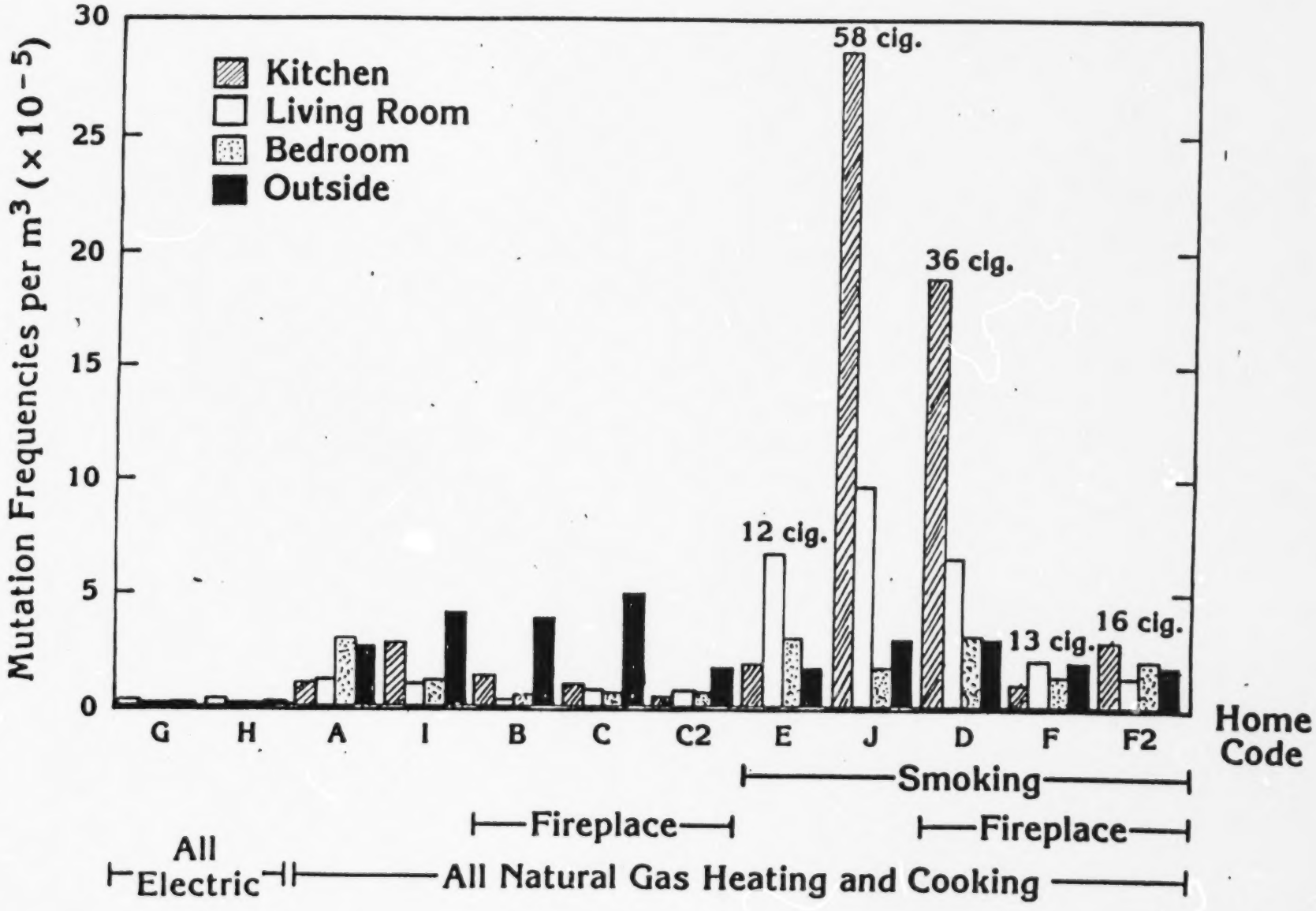

Mutation Frequencies per m³ (×10⁻⁵)
30
25
20
15
10
5
0
Kitchen
Living Room
Bedroom
Outside
58 cig.
36 cig.
12 cig.
13 cig.
16 cig.
G
H
A
I
B
C
C2
E
J
D
F
F2
Home Code
Smoking
Fireplace
Fireplace
All Electric
All Natural Gas Heating and Cooking

Fig. 5

Contribution of Source Emissions to Mutagenic Activity in the USA (revertants/year)

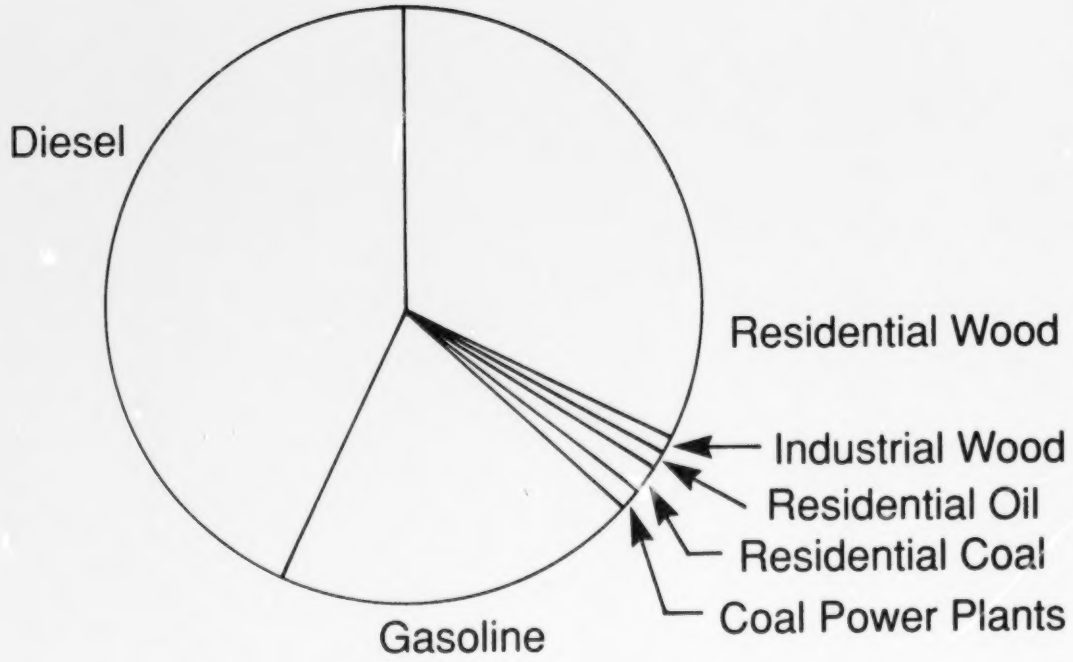
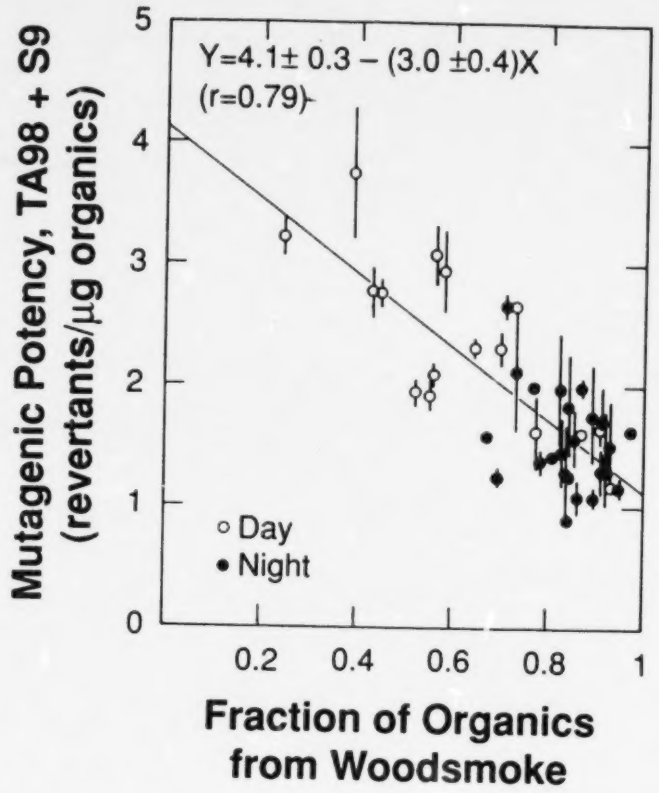


Fig. 6



Source Contribution to the Concentration of Mutagenic Activity

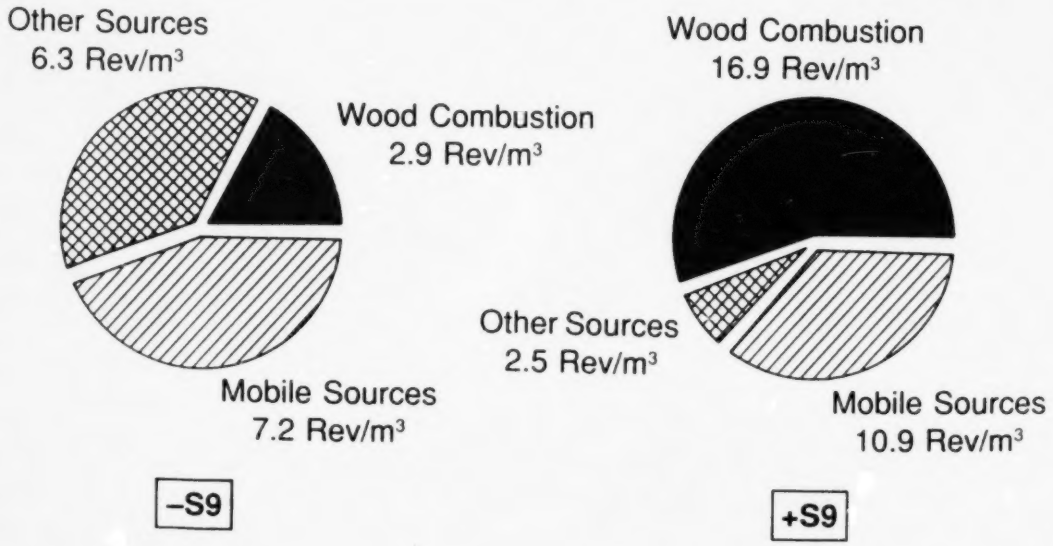


Fig. 7